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(71) Applicant: VERTEX PHARMACEUTICALS INCORPORATED [US/US]; 40 Allston Street, Cambridge, MA 02139 (US).

(72) Inventors: ARMISTEAD, David, M. ; 5 Cutting Drive, Maynard, MA 01754 (US). BOGER, Joshua, S. ; 243 Old Pickard Road, Concord, MA 01742 (US). MEYERS, Harold, V. ; 46 Van Ness Road, Belmont, MA 02178 (US). SAUNDERS, Jeffrey, O. ; 164 Parker Street, Acton, MA 01720 (US). TUNG, Roger, D. ; 2561 Massachusetts Avenue, No. 2, Cambridge, MA 02140 (US).

(74) Agents: OLEK, Alice, C. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

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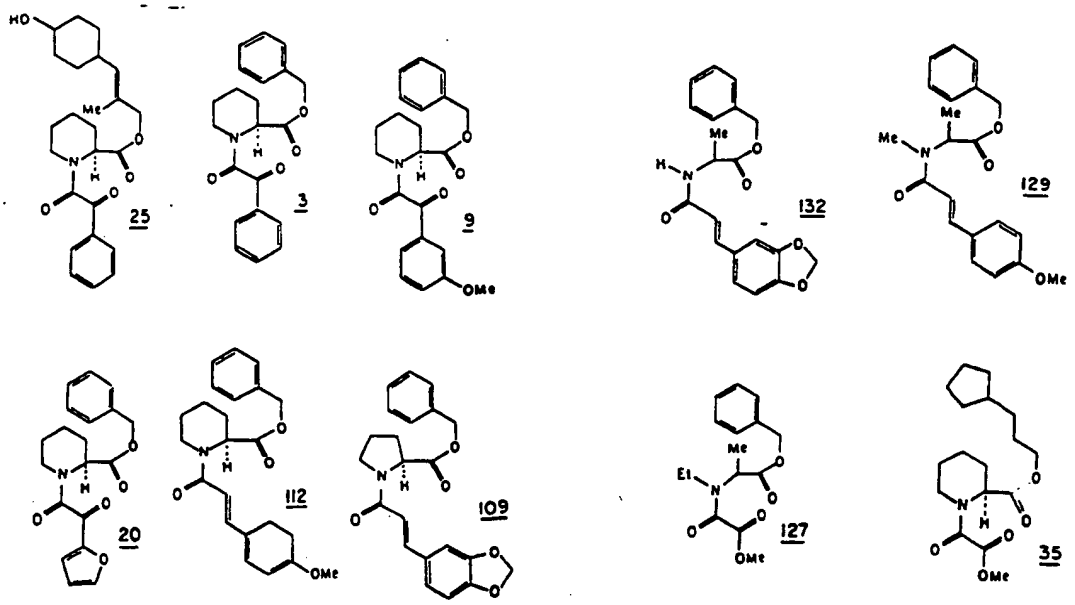

(57) Abstract

This invention relates to a novel class of immunosuppressive compounds having an affinity for the FK-506 binding protein (FKBP). Once bound to this protein, the immunosuppressive compounds inhibit the prolyl peptidyl cis-trans isomerase (rotamase) activity of the FKBP and inhibit T cell activation. As such, the compounds of this invention can be used as immunosuppressive drugs to prevent or significantly reduce graft rejection in bone marrow and organ transplantations and for use in the treatment of a wide variety of autoimmune diseases in humans and other mammals.

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NOVEL IMMUNOSUPPRESSIVE COMPOUNDS

Background of the Invention

Post operative graft rejections are a major complication affecting the success of bone marrow and organ transplantations. However, through the use of immunosuppressive drug therapy, graft rejection in organ transplantation can be significantly reduced.

A wide variety of diseases can be characterized as "autoimmune diseases". Such diseases are similar to graft rejection, except that the rejection is of self tissue. Immunosuppressive therapy can also be of use in preventing this inappropriate self rejection.

One widely accepted immunosuppressant for the prevention of graft rejection is cyclosporin A (CsA). It is a natural product of fungal metabolism and has been demonstrated to have potent immunosuppressive activity in clinical organ transplantations. Calne, R.Y. et al., Br. Med. J. 282:934-936 (1981); White, D.J.C. Drugs 24:322-334 (1982). Although CsA is widely used in immunosuppressant therapy, its usage (particularly in high dosage) is often accompanied by side effects which include nephrotoxicity, hepatotoxicity and other central nervous system disorders.

The following diseases have been treated with cyclosporin A with positive results, confirming the importance of the autoimmune component in these diseases and their effective treatment with compounds working by selective T-cell immune suppression similar to cyclosporin A.

1) Ophthalmology: Uveitis, Behcet's disease and Grave's ophthalmopathy.
Weetman, A.P. et al., Lancet 486-489 (1982). Grave's ophthalmopathy.
Nussenblatt, R.B. et al., Lancet 235-238 (1983). Uveitis.
French-Constant, C. et al., Lancet 454 (1983). Behcet's disease.
Sanders, M. et al., Lancet 454-455 (1983). Behcet's disease.
Note: Cyclosporin A is currently approved in Japan for the treatment of Behcet's disease, the first autoimmune disease indication for this compound.

2) Dermatology: Various autoimmune skin diseases including psoriasis.
Zabel, P. et al., Lancet 343 (1984). Acute dermatomyositis.
van Joost, T. et al., Arch. Dermatol. 123:166-167 (1987). Atopic skin disease.
Appleboom, T. et al., Amer. J. Med. 82:866-867 (1987). Scleroderma.
Logan, R.A. and R.D.R. Camo, J. Roy. Soc. Med. 81:417-418 (1988). Eczema.
Griffiths, C.E.M. et al., Brit. Med. J. 293:731-732 (1986). Psoriasis.
Ellis, C.N. et al., J. Amer. Med. Assoc. 256:3110-3116 (1986). Psoriasis.

3) Hematology: Various diseases including anemia.
Toetterman, T.H. et al., Lancet, 693 (1984). Pure red cell aplasia (PRCA).

Stryckmans, P.A. et al., New Engl. J. Med. 310:655-656 (1984). Aplastic anemia.

Gluckman, E. et al., Bone Marrow Transplant 3 Suppl. 1, 241 (1988). Aplastic anemia.

4) Gastroenterology/Hepatology: Primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease and other gastrointestinal autoimmune diseases.

Wiesner, R.H. et al., Hepatology 7:1025, Abst. #9, (1987). Primary biliary cirrhosis.

Hyams, J.S. et al., Gastroenterology 93:890-893 (1987). Autoimmune hepatitis.

Allison, M.C. et al., Lancet, 902-903 (1984). Crohn's disease.

Brynskov, J. et al., Gastroenterology 92:1330 (1987). Crohn's disease.

Porro, G.B. et al., Ital. J. Gastroenterol. 19:40-41 (1987). Ulcerative colitis.

5) Neurology: Amyotrophic lateral sclerosis (ALS, "Lou Gehrig's disease"), myasthenia gravis and multiple sclerosis.

Appel, S.H. et al., Arch. Neurol. 45:381-386 (1988). ALS.

Tindall, R.S.A. et al., New Engl. J. Med. 316:719-724 (1987). Myasthenia gravis.

Ann. Neurol. 24, No. 1, p. 169,m Abstract P174 (1988). Multiple sclerosis.

Dommasch, D. et al., Neurology 38 Suppl. 2, 28-29 (1988). Multiple sclerosis.

6) Nephrotic Syndrome: Nephrotic syndrome, membrano-proliferative glomerulonephritis (MPGN) and related diseases.

Watzon, A.R. et al., Clin. Nephrol. 25:273-274 (1986). Nephrotic syndrome.

Tejani, A. et al., Kidney Int. 33:729-734 (1988). Nephrotic syndrome.

Meyrier, A. et al., Transplat Proc. 20, Suppl. 4 (Book III), 259-261 (1988). Nephrotic syndrome.

LaGrue, G. et al., Nephron. 44:382-382 (1986). MPGN.

7) Rheumatoid Arthritis (RA)

Harper, J.I. et al., Lancet 981-982 (1984). RA

Van Rijthoven, A.W. et al., Ann. Rheum. Dis. 45:726-731 (1986). RA.

Dougados, M. et al., Ann. Rheum. Dis. 47:127-133 (1988). RA.

8) Insulin-Dependent Diabetes Mellitus (IDDM)

Stiller, C.R. et al., Science 223:1362-1367 (1984). IDDM.

Assan, R. et al., Lancet, 67-71 (1985). IDDM.

Bougneres, P.F. et al., New Engl. J. Med. 318:663-670 (1988). IDDM.

Diabetes 37:1574-1582 (1988). IDDM.

Many veterinary diseases are also characterized as autoimmune diseases. Autoimmune diseases such as those listed above have been observed in mammals. Papa, F.O. et al., Equine Vet. J. 22:145-146 (1990) infertility of autoimmune origin in the stallion; Gorman, N.T. and L.L. Werner, Brit. Vet. J. 142:403-410, 491-497 and

498-505 (1986) immune mediated diseases of cats and dogs; George, L.W. and S.L. White, Vet. Clin. North Amer. 6:203-213 (1984) autoimmune skin diseases in large mammals; Bennett, D., In. Pract. 6:74-86 (1984) autoimmune diseases in dogs; Halliwell, R.E., J. Amer. Vet. Assoc. 181:1088-1096 (1982) autoimmune diseases in domesticated animals.

The mechanism by which CsA causes immunosuppression has been established. In vitro, CsA inhibits the release of lymphokines, such as interleukin 2 (IL-2) [Bunjes, D. et al., Eur. J. Immunol. 11:657-661 (1981)] and prevents clonal expansion of helper and cytotoxic T cells [Larsson, E. J. Immunol. 124:2828-2833 (1980)]. CsA has been shown to bind the cytosolic protein, cyclophilin, and inhibit the prolyl-peptidyl cis-trans isomerase (PPIase) activity of that protein. Fischer, G. et al., Nature 337:476-478 (1989); Takahashi, N. et al., Nature 337:473-475 (1989). The PPIases may mediate T cell activation by catalyzing the rotomerization of peptide bonds of prolyl residues.

Recently, a second natural product isolated from Streptomyces, referred to as FK-506, has been demonstrated to be a potent immunosuppresive agent. Tanaka, H. et al., J. Am. Chem. Soc. 109:5031-5033 (1987). FK-506 inhibits IL-2 production, inhibits mixed lymphocyte culture response and inhibits cytotoxic T-cell generation in vitro at 100 times lower concentration than cyclosporin A. Kino, T. et al., J. Antibiot. 15:1256-1265 (1987). FK-506 also inhibits PPIase activity, but is structurally different from CsA and binds to a binding

protein (FKBP) distinct from cyclophilin. Harding, M.W. et al., Nature 341:758-760 (1989); Siekierka, J.J., Nature 341:755-757 (1989).

Summary of the Invention

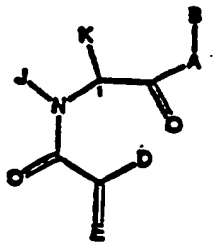
This invention relates to a novel class of immunosuppressive compounds having an affinity for the FK-506 binding protein (FKBP). Once bound to this protein, the immunosuppressive compounds inhibit the prolyl peptidyl cis-trans isomerase (rotamase) activity of the FKBP and lead to inhibition of T cell activation. The compounds of this invention can be used as immunosuppressive drugs to prevent or significantly reduce graft rejection in bone marrow and organ transplantations and in the treatment of autoimmune disease in humans and other mammals.

Brief Description of the Figure

Figure 1 illustrates some preferred compounds of this invention. The synthesis of each of the preferred compounds is described in detail in the Example section.

Detailed Description of the Invention

This invention relates to a novel class of immunosuppressive compounds represented by the formula I:

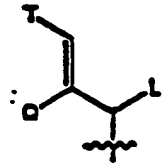


I

and pharmaceutically acceptable salts thereof,

wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B is hydrogen, CHL-Ar, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl or Ar substituted (C1-C6)-alkyl or alkenyl, or



wherein L and Q are independently hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, CF_3, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino and phenyl;

wherein D is either hydrogen or U; E is either oxygen or CH-U, provided that if D is hydrogen, then E is CH-U or if E is oxygen then D is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl or (C5-C7) -cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl)]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2 substituent therein.

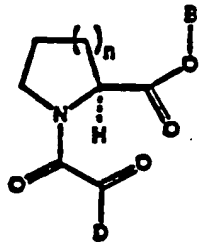
The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts

such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

Preferably, the compounds will have a molecular weight below about 750 atomic mass units (a.m.u.) and most preferably below about 500 a.m.u. Examples of compounds in which the J and K substituents are taken together to form a heterocyclic ring are shown in Tables 1 and 2. Examples of other preferred compounds of this invention are listed in Tables 3 and 4.

TABLE 1



No.	B	D	n	Ki	K_d
2	Benzyl	Phenyl	1	25μM	>5.0μM
3	Benzyl	Phenyl	2	1.5μM	>2.0μM
4	Allyl	Phenyl	2	8μM	ND
5	1-Naphthyl	Phenyl	2	0.9μM	ND
6	2-Naphthyl	Phenyl	2	7.0μM	1.0 μM
7	Benzyl	2-Methylpropyl	2	0.9μM	ND
8	Benzyl	2-Methoxyphenyl	2	17μM	>75μM
9	Benzyl	3-Methoxyphenyl	2	0.3μM	>1.3μM
10	Benzyl	4-Methoxyphenyl	2	5.0μM	5.0μM
11	Benzyl	3,5-Dimethoxyphenyl	2	2.0μM	0.6μM
12	Benzyl	2,6-Dimethoxyphenyl	2	50 μM	25μM
13	Benzyl	3,4,5-Trimethoxy-phenyl	2	0.1μM	2μM
14	Benzyl	4-Fluorophenyl	2	4.0μM	10μM
15	Benzyl	3-Nitrophenyl	2	160μM	>75μM
16	Benzyl	4-Nitrophenyl	2	160μM	100μM
17	Benzyl	2-Pyridyl	2	130μM	>1000μM
18	Benzyl	2-Pyridyl-N-oxide	2	>500μM	10μM
19	tert-Butyl	2-Furyl	1	200μM	>500μM

TABLE 1 (continued)

No.	B	D	n	Ki	K_d
20	Benzyl	2-Furyl	2	3μM	>12μM
21	Benzyl	3-Indolyl	2	25μM	20μM
22	Benzyl	2-Thiophenyl	2	0.8μM	4μM
23	E-3-Phenyl-2-methyl-prop-2-enyl	Phenyl	2	1.5μM	ND
24	E-3-(4-Hydroxy-phenyl)-2-methyl-prop-2-enyl	Phenyl	2	6μM	ND
25	E-3-[cis-(4-hydroxycyclo-hexyl)]-2-methyl-prop-2-enyl	Phenyl	2	0.6μM	ND
26	E-3-[trans-(4-Hydroxycyclo-hexyl)]-2-methyl-prop-2-enyl	Phenyl	2	0.5μM	ND
27	Benzyl	2-Nitrobenzyl	1	26μM	>25μM
28	Hydrogen	Methoxy	2	ND	ND
29	tert-Butyl	Methoxy	1	600μM	>500μM
30	Allyl	Methoxy	2	190μM	>25μM
31	Benzyl	Methoxy	2	80μM	>50μM
32	2-Cyclohexylethyl	Methoxy	2	45μM	>40μM
33	3-Cyclohexylpropyl	Methoxy	2	20μM	12μM
34	4-Cyclohexylbutyl	Methoxy	2	6μM	2-3μM

TABLE 1 (continued)

No.	B	D	n	Ki	K_d
35	Cyclopentyl-propyl	Methoxy	2	35μM	ND
36	E-3-(4-Methoxy-phenyl)-2-methyl-prop-2-enyl	Methoxy	2	40μM	>30μM
37	E-3-(3,4-Dimethoxyphenyl)-2-methyl-prop-2-enyl	Methoxy	2	10μM	ND
38	E-3-(4-Hydroxy-phenyl)-2-methyl-prop-2-enyl	Methoxy	2	60μM	ND
39	E-3-[cis-(4-Hydroxycyclohexyl)]-2-methyl-prop-2-enyl	Methoxy	2	70μM	>20μM
40	Benzyl	Cyclohexyl	2	1.3μM	3μM
41	Benzyl	Ethyl	1	400μM	>300μM
42	Benzyl	3-Methoxyphenyl	1	5 μM	80 μM
43	Benzyl	2-Pyridyl	1	300 μM	ND
44	Benzyl	3,4-Difluorophenyl	2	3 μM	ND
45	Benzyl	(*E*)-2-(4-Methoxyphenyl)-ethenyl	2	1 μM	ND
46	Benzyl	1-Hydroxy-1-cyclohexyl	2	1 μM	2 μM
47	Benzyl	2-Naphthyl	2	1.5 μM	0.3 μM
48	Benzyl	1-Naphthyl	2	1 μM	2 μM
49	(*S*)-α-Methylbenzyl	Phenyl	2	0.5 μM	0.6μM
50	Benzyl	2-Hydroxy-2-tetrahydropyranyl	2	12 μM	0.35 μM

TABLE 1 (continued)

No.	B	D	n	Ki	K_d
51	(*R*)-α-Methylbenzyl	Phenyl	2	1.5 μM	1 μM
52	Benzyl	3-Trifluoromethylphenyl	2	1.5 μM	1.3 μM
53	Benzyl	3-Benzyloxyphenyl	2	0.5 μM	0.2 μM
54	Benzyl	(*E*)-2-*tert*-Butylethenyl	2	9 μM	3 μM
55	Benzyl	2-Trifluoromethylphenyl	2	5 μM	ND
56	4-Cyclohexylbutyl	Phenyl	2	0.4 μM	ND
57	4-Cyclohexylbutyl	3,4,5-Trimethoxyphenyl	2	0.04 μM	0.1 μM
58	4-Phenylbenzyl	Phenyl	2	5 μM	ND
59	4-Phenylbenzyl	3,4,5-Trimethoxyphenyl	2	2 μM	ND
60	Benzyl	3-Ethoxyphenyl	2	0.56 μM	ND
61	3-Phenoxybenzyl	3,4,5-Trimethoxyphenyl	2	0.018 μM	0.035 μM
62	3-Phenoxybenzyl	Phenyl	2	0.09μM	0.15 μM
63	4-Phenylbutyl	3,4,5-Trimethoxyphenyl	2	0.019 μM	0.1 μM
64	4-Phenylbutyl	Phenyl	2	0.35 μM	ND
65	Benzyl	3-(3-Propenyloxy)phenyl	2	1 μM	ND
66	Benzyl	3-(2-Propoxy)phenyl	2	0.5 μM	ND
67	Benzyl	1-Methylpropyl	2	1 μM	ND
68	2-Phenylethyl	Phenyl	2	1.1 μM	ND
69	6-Phenylhexyl	Phenyl	2	0.5 μM	ND
70	5-Phenylpentyl	3,4,5-Trimethoxyphenyl	2	0.07 μM	ND
71	6-Phenylhexyl	3,4,5-Trimethoxyphenyl	2	0.1 μM	0.05 μM
72	6-Cyclohexylhexyl	3,4,5-Trimethoxyphenyl	2	0.05 μM	0.5 μM
73	4-Phenoxybenzyl	3,4,5-Trimethoxyphenyl	2	0.8 μM	ND

TABLE 1 (continued)

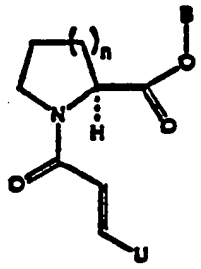
No.	B	D	n	Ki	K_d
74	5-Cyclohexylpentyl	3,4,5-Trimethoxyphenyl	2	0.09 μM	0.08 μM
75	Benzyl	3-(1-Butoxy)phenyl	2	0.36 μM	ND
76	4-Phenylbutyl	3-(2-Propoxy)phenyl	2	0.1 μM	ND
77	4-(4-Iodophenyl)butyl	3,4,5-Trimethoxyphenyl	2	0.016 μM	0.06 μM
78	4-Iodobenzyl	3,4,5-Trimethoxyphenyl	2	1.4 μM	ND
79	2-(2-Naphthyl)ethyl	3,4,5-Trimethoxyphenyl	2	0.22 μM	ND
80	2-(1-Naphthyl)ethyl	3,4,5-Trimethoxyphenyl	2	0.5 μM	ND
81	4-Phenylbutyl	4-Iodophenyl	2	0.8 μM	0.25 μM
82	4-Phenylbutyl	3-Iodophenyl	2	0.13 μM	0.2 μM
83	3-Phenylpropyl	3,4,5-Trimethoxyphenyl	2	0.11 μM	ND
84	3-(3-Indolyl)propyl	3,4,5-Trimethoxyphenyl	2	0.017 μM	0.054 μM
85	4-(4-Methoxyphenyl)butyl	3,4,5-Trimethoxyphenyl	2	0.013 μM	0.049 μM
86	4-Phenylbut-2-enyl	3,4,5-Trimethoxyphenyl	2	0.8 μM	ND
87	4-Phenylbut-3-enyl	3,4,5-Trimethoxyphenyl	2	0.5 μM	ND
88	4-(4-Allocaminophenyl)propyl	3,4,5-Trimethoxyphenyl	2	0.011 μM	0.07 μM
89	4-Phenylpropyl	1-Cyclohexenyl	2	0.78 μM	ND
90	4-(4-Methoxyphenyl)but-3-enyl	3,4,5-Trimethoxyphenyl	2	0.011 μM	0.60 μM
91	4-Phenylpropyl	1-Fluoro-1-cyclohexyl	2	0.54 μM	ND
92	4-Phenylpropyl	3-Butoxyphenyl	2	1.4 μM	ND
93	3-[3-(N-Formylindolyl)]propyl	3,4,5-Trimethoxyphenyl	2	0.015 μM	0.06 μM
94	4-(3-Indolyl)butyl	3,4,5-Trimethoxyphenyl	2	0.016 μM	0.05 μM
95	4-Phenylbutyl	Benzyl	2	0.35 μM	ND

TABLE 1 (continued)

No.	B	D	n	Ki	K_d
96	4-Phenylbutyl	3-Biphenyl	2	0.04 μM	0.033 μM
97	4-Phenylbutyl	4-*tert*-Butylphenyl	2	0.6 μM	ND
98	4-Phenylbutyl	Cyclohexyl	2	0.08 μM	0.18 μM
99	4-Phenylbutyl	Cyclohexylmethyl	2	0.12 μM	ND
100	4-Phenylbutyl	3,4-Methylenedioxyphenyl	2	0.25 μM	ND
101	4-Phenylbutyl	4-Tetrahydropyranyl	2	0.44 μM	ND
102	4-Phenylbutyl	3-Cyclohexyl-4-methoxy-phenyl	2	14 μM	ND
103	4-Phenylbutyl	4-(4-Methoxybenzyloxy-methyl)-2-furyl	2	0.7 μM	ND
104	4-Phenylbutyl	*tert*-Butyl	2	0.18 μM	ND
105	4-Phenylbutyl	Ethyl	2	1.6 μM	ND
106	3-(N-Benzimidazolyl)propyl	3,4,5-Trimethoxyphenyl	2	0.11 μM	ND
107	3-(N-Purinyl)propyl	3,4,5-Trimethoxyphenyl	2	0.13 μM	ND
108	(*S*,*S*)-2-Methyl-3-hydroxy-4-phenylpropyl	3,4,5-Trimethoxyphenyl	2	0.25 μM	ND

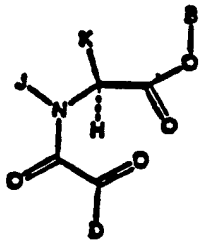
ND indicates not determined.

TABLE 2



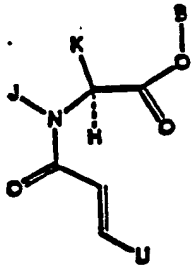
No.	B	U	n	Ki	K_d
109	Benzyl	3,4-Methylenedioxyphenyl	1	3μM	>15μM
110	Benzyl	3,4-Methylenedioxyphenyl	2	3μM	>4μM
111	Benzyl	4-Methoxyphenyl	1	6μM	>30μM
112	Benzyl	4-Methoxyphenyl	2	4μM	>8μM
113	Benzyl	2,5-Dimethoxyphenyl	1	10μM	ND
114	Benzyl	2,4,5-Trimethoxyphenyl	1	25μM	ND
115	Benzyl	3,4,5-Trimethoxyphenyl	1	450μM	>25μM
116	Benzyl	4-Dimethylaminophenyl	2	20μM	>5μM
117	Benzyl	4-Nitrophenyl	2	14μM	>5μM
118	Benzyl	2-Furyl	2	2.5μM	ND
119	Benzyl	3-Furyl	2	2.5μM	ND
120	Benzyl	3-Indolyl	2	>60μM	>8μM
121	Benzyl	3-Pyridyl	2	25μM	ND
122	Benzyl	Hydrogen	2	300μM	ND
123	Benzyl	Phenyl	2	11μM	ND

TABLE 3



No	B	D	J	K	Ki	K_d
124	Benzyl	Methoxy	Methyl	Hydrogen	1000μM	>200μM
125	Benzyl	Methoxy	Methyl	S-Methyl	400μM	>200μM
126	Benzyl	Methoxy	Methyl	S-Isopropyl	170μM	>200μM
127	Ethyl	Methoxy	Benzyl	Hydrogen	>1200μM	>300μM
128	tert-Butyl	Methoxy	Ethyl	S-Methyl	>400μM	>500μM

TABLE 4



No	B	U	J	K	Ki	K_d
129	Benzyl	4-Methoxy-phenyl	Methyl	S-Methyl	80μM	>150μM
130	Benzyl	4-Methoxy-phenyl	Methyl	S-Isopropyl	30μM	>20μM
131	Benzyl	3,4-Methylene-dioxyphenyl	Methyl	S-Methyl	50μM	ND
132	Benzyl	3,4-Methylene-dioxyphenyl	Hydrogen	S-Methyl	60μM	ND

The immunosuppressive compounds of this invention have an affinity for the FK-506 binding protein which is located in the cytosol of lymphocytes, particularly T lymphocytes. When the immunosuppressive compounds are bound to the FKBP, they act to inhibit the prolyl-peptidyl cis-trans isomerase activity of the binding protein and inhibit lymphocyte activation mediated by FKBP. One particular FK-506 binding protein has been identified by Harding, M.W. et al., Nature 341:758-760 (1989) and can be used as the standard by which to evaluate binding affinity of the compounds for FKBP. Compounds of this invention, however, may have an affinity for other FK-506 binding proteins. Inhibition of the prolyl peptidyl cis-trans isomerase may further be indicative of binding to an FK-506 binding protein.

Human FK-506 binding protein can be obtained as described by Harding, M.W. et al., Nature 341:758-760 (1989). Values for the apparent K_d can be determined from a competitive LH-20 binding assay performed as described by Harding et al., using 32-[1-^{14}C]-benzoyl FK-506 as a reporting ligand; or using [^{3}H]dihydro-FK-506, as described by Siekierka, J.J. et al., Nature 341:755-757 (1989). The binding affinities for several compounds of this invention for the FKBP are reported in Tables 1-4. The data was obtained using the latter method, where the ability of an unlabeled compound to compete with the binding of [^{3}H]dihydro-FK-506 to FK-506 binding protein was measured.

The inhibition of the PPIase (rotamase) enzyme activity of the FKBP (apparent "Ki" values) can also be measured according to the methods described by either

Harding, M.W. et al., Nature 341:758-760 (1989) or Siekierka, J.J. et al., Nature 341:755-757 (1989). The cis-trans isomerization of the proline-alanine peptide bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, is monitored spectrophotometrically in a coupled assay with chymotrypsin, which releases 4-nitroanilide from the trans form of the substrate. Fischer, G. et al., Nature 337:476-478 (1989). The inhibitory effect of the addition of different concentrations of inhibitor on the extent of the reaction is determined, and analysis of the change in first order rate constant as a function of inhibitor concentration yields an estimate of the apparent Ki value. The extent of enzyme inhibition (K_i) of some preferred compounds is shown in Tables 1-4.

The compounds of the present invention can be further characterized in cellular biological experiments in vitro where their resemblance in function and use to cyclosporin A and to FK-506 is apparent. (See Tables 5 and 6)

TABLE 5

Assays and IC_{50} Value for Drugs	Cyclosporin A	Rapamycin	FK-506
1) Human PBL + OKT3	<1μg/ml	<1μg/ml	<1μg/ml
2) T-Cell Hybridoma + TCR/CD2	<1μg/ml	<1μg/ml	<1μg/ml
3) Apoptosis	Blocks at 1μg/ml	Inactive at 1μg/ml	Blocks at 1μg/ml
4) CTLL Proliferation + IL-2	>>1μg/ml	≈0.01μg/ml	>>1μg/ml

TABLE 6: Cellular Assay Results

No.	PMA (μM)	OKT3 (μM)	LB (μM)	JVM (μM)	CTLL (μM)
2	ND	ND	ND	ND	ND
3	7.6	4.6	>10	>10	>8.5
4	ND	ND	ND	ND	ND
5	>10	>10	>10	>10	>10
6	ND	ND	ND	ND	ND
7	>10	>10	>10	>10	>10
8	ND	ND	ND	ND	ND
9	>10	6.5	>10	>10	>10
10	ND	ND	ND	ND	ND
11	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND
13	>10	5.9	>10	>10	>10
14	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND
16	>10	>10	>10	>10	>10
17	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND
19	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND

TABLE 6 (Cont.)

21	ND	ND	ND	ND	ND
22	>10	>10	>10	>10	>10
23	>10	>10	>10	>10	>10
24	ND	ND	ND	ND	ND
25	>10	>10	>10	>10	>10
25	>10	>10	>10	>10	>10
26	>10	6.5	>10	>10	>10
27	ND	ND	ND	ND	ND
28	ND	ND	ND	ND	ND
29	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND
31	ND	ND	ND	ND	ND
32	ND	ND	ND	ND	ND
33	ND	ND	ND	ND	ND
34	ND	ND	ND	ND	ND
35	ND	ND	ND	ND	ND
36	ND	ND	ND	ND	ND
37	ND	ND	ND	ND	ND
38	ND	ND	ND	ND	ND
39	ND	ND	ND	ND	ND
40	7.0	1.0	>10	>10	>10

TABLE 6 (Cont.)

41	ND	ND	ND	ND	ND
42	ND	ND	ND	ND	ND
43	ND	ND	ND	ND	ND
44	ND	ND	ND	ND	ND
45	>10	>10	>10	>10	>10
46	>10	>10	>10	>10	>10
47	>10	>10	>10	>10	>10
48	>10	>10	>10	>10	>10
49	>10	6.2	>10	>10	>10
50	ND	ND	ND	ND	ND
51	ND	ND	ND	ND	ND
52	>10	>10	>10	>10	>10
53	>10	8.0	>10	>10	8.0
54	ND	ND	ND	ND	ND
55	ND	ND	ND	ND	ND
56	>10	>10	>10	6.5	5.0
57	4.0	4.5	>10	8.0	6.0
58	ND	ND	ND	ND	ND
59	ND	ND	ND	ND	ND
60	>10	>10	>10	>10	>10

TABLE 6 (Cont.)

61	4.0	8.0	>10	>10	3.2
62	6.5	>10	>10	>10	>10
63	6.0	3.1	10	8.5	3.8
64	10	6.0	>10	>10	>10
65	>10	>10	>10	>10	>10
66	>10	>10	>10	>10	>10
67	>10	>10	>10	>10	>10
68	>10	>10	>10	>10	>10
69	6.1	>10	>10	>10	>10
70	7.0	4.5	>10	9.0	4.2
71	5.0	5.5	7.5	8.0	3.8
72	9.0	>10	>10	>10	5.5
73	8.0	>10	4.5	6.0	7.0
74	8.0	9.0	10	10	5.0
75	8.0	>10	9.0	>10	4.5
76	5.0	10	9.0	>10	6.0
77	4.5	8.0	6.0	7.0	2.1
78	>10	>10	>10	>10	>10
79	10	2.5	>10	>10	8.0
80	3.0	4.0	10	10	6.0

TABLE 6 (Cont.)

81	7.0	>10	>10	>10	>10
82	10	>10	>10	>10	>10
83	5.5	5.5	8.5	7.5	5.0
84	4.5	6.0	6.0	>10	2.0
85	4.5	4.5	7.0	10	1.5
86	9.0	>10	>10	>10	>10
87	7.0	>10	>10	>10	>10
88	2.2	2.2	2.5	4.5	4.0
89	8.0	>10	>10	>10	>10
90	8.0	>10	>10	>10	7.0
91	>10	>10	>10	>10	>10
92	9.0	>10	>10	>10	>10
93	6.0	7.0	10	8.7	3.7
94	5.0	5.5	>10	7.0	4.0
95	>10	>10	>10	>10	>10
96	>10	>10	>10	>10	>10
97	>10	>10	>10	>10	6.0
98	>10	>10	>10	>10	>10
99	>10	>10	>10	>10	>10
100	>10	>10	>10	>10	>10
101	>10	>10	>10	>10	>10
102	>10	>10	10	10	>10

TABLE 6 (Cont.)

103	7.0	10	10	>10	10
104	4.0	>10	>10	>10	>10
105	>10	>10	>10	>10	>10
106	7.0	>10	>10	>10	3.0
107	6.5	>10	>10	>10	>10
108	>10	>10	8.0	>10	>10
109	ND	ND	ND	ND	ND
110	ND	ND	ND	ND	ND
111	ND	ND	ND	ND	ND
112	ND	ND	ND	ND	ND
113	ND	ND	ND	ND	ND
114	ND	ND	ND	ND	ND
115	ND	ND	ND	ND	ND
116	ND	ND	ND	ND	ND
117	ND	ND	ND	ND	ND
118	ND	ND	ND	ND	ND
119	ND	ND	ND	ND	ND
120	ND	ND	ND	ND	ND
121	ND	ND	ND	ND	ND
122	ND	ND	ND	ND	ND

TABLE 6 (Con.)

123	ND	ND	ND	ND	ND
124	ND	ND	ND	ND	ND
125	ND	ND	ND	ND	ND
126	ND	ND	ND	ND	ND
127	ND	ND	ND	ND	ND
128	ND	ND	ND	ND	ND
129	ND	ND	ND	ND	ND
130	ND	ND	ND	ND	ND
131	ND	ND	ND	ND	ND
132	ND	ND	ND	ND	ND

All of the compounds in Table 6 showed toxicity at higher concentrations than their immunosuppresive activity and were typically concentrations > 10 μM.

PMA and OKT3 - mitogens used to stimulate proliferation of human peripheral blood lymphocytes (PBC). Compounds are evaluated on their ability to inhibit proliferation.

LB and JVM - human viral-transformed B lymphoblastoid cell lines stimulated to proliferate in a mixed lymphocyte reaction (MLR). The compounds are evaluated on their ability to inhibit this proliferation.

CTLL - inhibition of proliferation of cytotoxic T cells stimulated by IL-2.

1) Assay similar to Yoshimura, N. et al., Transplantation 47:356-359 (1989). Assay uses fresh human peripheral blood lymphocytes isolated by Ficoll-Hypaque density centrifugation, stimulated by the OKT3 antibody (anti-CD3) which stimulates via interaction with CD3. Stimulation is measured by incorporation of radioactive thymidine [(^{3}H)TdR] into proliferating cells, with an uninhibited control signal of 48,000-75,000 cpm. IC_{50} values are estimated from inhibitions of proliferation observed at various drug concentrations.

2) Assay similar to above, but using T-cell clone stimulated with antibody to the T-cell receptor (TCR) and antibody to CD2. Stimulation is measured by incorporation of radioactive thymidine [(^{3}H)TdR] into proliferating cells, with an uninhibited control signal of 23,000 cpm. IC_{50} values are estimated from inhibitions of proliferation observed at various drug concentrations.

3) Assay according to Shi, Y. et al., Nature 339:625-626 (1989). The assay uses a T-cell hybridoma similar to that described. The assay measures activation-induced (anti-CD3) cell death (evaluated by counting viable cells after staining as described) in a T-cell hybridoma that mimics the effect known to occur in immature thymocytes. The ability of cyclosporin A and FK-506 to inhibit this cell death is herein used as a sensitive indication of compounds with cyclosporin-like and/or FK-506-like mechanism of action. Note that the chemically related, but mechanistically distinct, immunosuppressant rapamycin is inactive in this assay.

4) Assay according to DuMont, F. et al., J. Immunol. 144:251-258 (1990). The assay measures the stimulation of CTLL cells in response to IL-2. Proliferation is measured by incorpoation of (^{3}H)TdR. Immunosuppressants which work by a similar mechanism to cyclosporin A and FK-506 will not inhibit in this IL-2 driven process, since they function by the inhibition of production of endogenous IL-2. In this assay, exogenous IL-2 is provided to overcome this block. Note that the chemically related, but mechanistically distinct immunosuppressant, rapamycin, is active in this assay.

These assays can be used to profile the cellular activity of the compounds of the present invention. Thus, it is clear from these results that the compounds resemble both cyclosporin A and FK-506 in its cellular activity, including immunosuppression, in contrast to the mechanistically dissimilar immunosuppressant agent rapamycin. Furthermore, the observed cellular activity is consistent quantitatively with the activity observed for FKBP binding and inhibition of PPIase (rotamase) activity shown in Table 1.

Thus, the compounds can be used as immunosuppressants for prophylaxis of organ rejection or treatment of chronic graft rejection and for the treatment of autoimmune diseases.

The immunosuppressive compounds of this invention can be periodically administered to a patient undergoing bone marrow or organ transplantation or for another reason in which it is desirable to substantially reduce or suppress a patient's immune response, such as in

various autoimmune diseases. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian autoimmune diseases.

The novel compounds of the present invention possess an excellent degree of activity in suppression of antigen-stimulated growth and clonal expansion of T-cells, especially those T-cells characterized as "helper" T-cells. This activity is useful in the primary prevention of organ transplant rejection, in the rescue of transplanted organs during a rejection episode, and in the treatment of any of several autoimmune diseases known to be associated with inappropriate autoimmune responses. These autoimmune diseases include: uveitis, Behcet's disease, Graves ophthalmopathy, psoriasis, acute dermatomyositis, atopic skin disease, scleroderma, eczema, pure red cell aplasia, aplastic anemia, primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease, amyotrophic lateral sclerosis, myasthenia gravis, multiple sclerosis, nephrotic syndrome, membranoproliferative glomerulonephritis, rheumatoid arthritis and insulin-dependent diabetes mellitus. In all of the above-listed autoimmune diseases, treatment is effective to reduce the symptoms and slow progression of the disease. In the case of insulin-dependent diabetes mellitus, treatment as described below is most effective when instituted before the complete cessation of natural insulin production and transition to complete dependence on external insulin.

For these purposes the compounds of the present invention may be administered orally, parenterally, by

inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrasternal and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectible aqueous or oleagenous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, as do natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or similar alcohol.

The compounds may be administered orally, in the form of capsules or tablets, for example, or as an

aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including autoimmune diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a

preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses, the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of 0.01 to 100 mg/kg per day of the active ingredient compound are useful in the treatment of the above conditions. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate

of excretion, drug combination and the severity of the particular disease being treated.

The compound can also be administered in combination with a steroid, such as methyl prednisalone acetate, for additional immunosuppressive effect. The steroid is administered orally, intravenously, rectally, topically or by inhalation. Dosages (based upon methyl prednisalone acetate) of 0.1-5 mg/kg/day may be employed. An initial loading dose of 100-500 mg may be employed. Steroid doses may be decreased with time from the higher toward the lower doses as the clinical situation indicates.

The compounds can be administered with other immunosuppressant drugs, such as rapamycin, azathioprine, 15-deoxyspergualin, cyclosporin, FK-506 or combinations of these, to increase the immunosuppressive effect. Administration of cyclosporin and FK-506 together should be avoided due to contraindications reported resulting from coadministration of these immunosuppressants. The dosage level of other immunosuppressant drugs will depend upon the factors previously stated and the immunosuppressive effectiveness of the drug combination.

OKT3, which is a murine monoclonal antibody to CD3 surface antigen of human T lymphocytes, can also be coadministered intravenously with compounds of the present inventions for rescue and reversal of acute allograft rejections, particularly in renal transplantations.

The invention will be further illustrated by way of the following examples, which are not intended to be limiting in any way.

EXAMPLES

General

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz on a Bruker AC 300 or at 500 MHz on a Bruker AMX 500. Chemical shifts for proton resonances are reported in parts per million (δ) relative to Me_4Si (δ 0.0). Analytical high performance liquid chromatography (HPLC) was performed on either a Waters 600 E or a Hewlett Packard 1050 liquid chromatograph. HPLC assessments of compounds were run on a Waters Associates Delta Pak 5 micron, 15 cm. column at a flow rate of 1.5 mL per minute. The solvent system used was: A = 0.1% H_3PO_4/H_2O; B = 0.1% H_3PO_4/CH_3CN. A linear gradient of 95% A/5% B to 100% B over 15 minutes followed by 1.5 minutes at 100% B was used, with detection at 214 nM.

The compounds described below are illustrated in Figure 1.

EXAMPLE 1

Synthesis of (E)-3-[cis-4-(hydroxycyclohexyl)]-2-methylprop-2-enyl N-(phenylglyoxyl)-pipecolate (25).

1. (S)-Benzyl Pipecolate (133)

To a slurry of 7.3 g (26.14 mmol) of the tartrate salt of (S)-pipecolic acid (Egbertson M. and S.J. Danishefsky, J. Org. Chem. 54:11 (1989) in 75 mL of dry benzene was added 13.5 mL (0.13 mol) of benzyl alcohol and 5.48 g (28.8 mmol) of p-toluenesulfonic acid monohydrate. The reaction mixture was heated at reflux

under a Dean-Stark trap for 2 h and then cooled to room temperature. The solution was then diluted with 400 mL of ether and stirred overnight at 4°C. The resulting white solid was collected on a filter, washed with hexane and dried in vacuo to give 9.2 g (90%) of the p-toluene-sulfonic acid salt of benzyl pipecolate (134). ^{1}H NMR (300 MHz, D_2O) δ 7.63 (d), 7.41 (s), 7.28 (d), 5.26 (ABq), 4.8 (s), 4.03 (dd), 3.92 (dd), 3.51-3.39 (m), 3.15-2.93 (s), 2.40 (s), 2.36-2.24 (m), 1.98-1.53 (m).

Benzyl pipecolate was routinely generated by treating an ethyl acetate suspension of this salt with saturated sodium bicarbonate until dissolution of the organic material. The aqueous layer was extracted twice with ethyl acetate and the combined organic extracts were dried with $MgSO_4$ and evaporated to yield (S)-Benzyl pipecolate (133) as a pale yellow oil.

2. (S)-N-(Phenylglyoxyl)pipecolic Acid (135)

To a solution of 4.95 g (17.72 mmol) of the tartrate salt of L-(S)-pipecolic acid in 18.0 mL of methylene chloride at 0°C was added 20.4 mL (117.10 mmol) of diisopropylethylamine followed by 12.4 mL (97.7 mmol) of chlorotrimethylsilane and the resulting solution was stirred at 0°C for 15 minutes. To this mixture was added 17.72 mmol of benzoylformyl chloride, which was freshly prepared in a separate reaction flask at ambient temperature from 2.66 g (17.72 mmol) of benzoylformic acid and 2.3 mL (26.37 mmol) of oxalyl chloride in 18.0 mL of methylene chloride containing a catalytic amount of dimethylformamide. The reaction mixture was stirred at 25°C overnight at which time the mixture was poured into

1.0 N HCl. The aqueous layer was discarded and the organic layer was washed twice with saturated sodium bicarbonate. The combined aqueous layers were washed with methylene chloride, acidified to pH 2.0 with concentrated HCl, and then extracted repeatedly with ether. Flash chromatography (Still, W.C. et al., J. Org. Chem. 43:2923 (1978)) (elution with 1:1 ethyl acetate-hexane containing 1% acetic acid provided 2.3 g of (S)-N-(Phenylglyoxyl)-pipecolic acid (135) as a rotameric mixture. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.4-11.1 (br s), 8.02 (d), 7.98 (d), 7.65 (t), 7.58-7.43 (m), 5.45 (d), 4.64 (dd), 4.43 (d), 3.52 (dd), 3.25 (ddd), 3.01 (ddd), 2.42 (d), 2.24 (d), 1.86-1.78 (m), 1.68-1.38 (m).

3. cis-and trans-4-(tert-Butyldimethylsilyloxy)-cyclohexane-1-ol (136) and (137)

To a solution of 3.43 g (21.7 mmol) of cis- and trans-methyl 4-hydroxycyclohexane carboxylate (Noyce, D.S. and D.B. Denney, J. Am. Chem. Soc. 74:5912 (1952)) in 45 mL of methylene chloride at 0°C was added 3.0 mL (26.0 mmol) of 2,6-lutidine followed by 5.5 mL (23.8) mmol of tert-butyldimethylsilyl trifluoromethanesulfonate. The ice bath was removed and the reaction mixture was allowed to stir at 25°C for 2 h at which time the solution was poured into saturated sodium bicarbonate. The layers were partitioned and the organic layer was washed with saturated copper sulfate and water and then dried over $MgSO_4$ to give 5.9 g of the crude methyl esters. A solution of 5.72 g (21.0 mmol) of this mixture in 45 mL of anhydrous THF was treated with 400 mg (10.5 mmol) of lithium aluminum hydride. The reaction

mixture was stirred at 25°C for 0.5 h and was then quenched by the slow addition of a saturated solution of Rochelle's salt. The mixture was diluted with ether, the layers were partitioned and the aqueous layer was washed twice with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and concentrated to give 4.9 g of the diastereomeric alcohols. Flash chromatography (elution with 1:5 ethyl acetate-hexane) gave 650 mg of (136), 1.10 g of (137) and 2.40 g of a mixture of the two. Data for (136): ^1H NMR (300 MHz, $CDCl_3$) δ 3.99-3.92 (m), 3.46 (d), 1.72-1.58 (m), 1.57-1.36 (m), 0.86 (s), 0.08 (s). Data for (137): ^1H NMR (300 MHz, $CDCl_3$) δ 3.47 (dddd), 3.38 (d), 1.86-1.67 (m), 1.47-1.16 (m), 1.05-0.77 (m), 0.72 (s), -0.02 (s).

4. <u>(E)-Ethyl 3-[cis-(4-tert-Butyldimethylsilyl-oxycyclohexyl)]-2-methylprop-2-enoate (138)</u>

To a -78°C solution of oxalyl chloride (465 μL, 5.33 mmol) in 5.0 mL of methylene chloride was added dimethylsulfoxide (755 μL, 10.65 mmol). The resulting solution was stirred for 5 min and then 650 mg (2.66 mmol) of the alcohol (136) was added in 5.0 mL of methylene chloride. The reaction mixture was stirred at -78°C for 45 min at which time 2.2 mL (16.0 mmol) of triethylamine was added and the solution was allowed to warm to ambient temperature. The reaction was quenched with 1.0 N HCl and the aqueous layer was extracted with three portions of methylene chloride. The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness to give 620 mg of the intermediate aldehyde which was treated

directly with 1.22 g (3.36 mmol) of (carbethoxyethylidine)triphenylphosphorane in 5.0 mL of methylene chloride. The resulting reaction mixture was stirred at ambient temperature overnight and was then poured into water. The layers were partitioned and the aqueous layer was extracted twice with methylene chloride. The combined organic layers were dried over $MgSO_4$ and concentrated to yield 1.55 g of crude product. Flash chromatography (elution with 1:20 ether-hexane) gave 300 mg of the enoate (138) as an oil.

5. (E)-3-[cis-(4-tert-Butyldimethylsilyloxycyclohexyl)]-2-methylprop-2-en-1-ol (139)

To a solution of 300 mg (0.95 mmol) of enoate (138) in 2.0 mL of anhydrous tetrahydrofuran at 25°C was added 18 mg (0.43 mmol) of lithium aluminum hydride and the resulting mixture was allowed to stir for 30 min. The reaction was quenched by the slow addition of saturated Rochelle's salt and diluted with ethyl acetate. The layers were separated and the aqueous layer was extracted with two portions of ethyl acetate. The combined organic extracts were washed with water and brine and then dried over $MgSO_4$. Evaporation and flash chromatography (elution with 1:10 ethyl acetate-hexane) gave 220 mg of the allyic alcohol (139). ^1H NMR (300 MHz, $CDCl_3$) δ 5.34 (d), 3.96 (d), 3.85 (m), 2.26-2.18 (m), 1.64 (d), 1.61-1.34 (m), 1.82 (s), 0.0 (s).

6. (E)-3-[cis-(4-tert-Butyldimethylsilyloxycyclohexyl)]-2-methylprop-2-enyl N-(phenylglyoxyl)-pipecolate (140)

To a solution of 68 mg (0.24 mmol) of allylic alcohol (139), 42.3 mg (0.16 mmol) of acid (135) and 39.8 mg (0.20 mmol) of 1-(3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (EDC) in 2.0 mL of anhydrous methylene chloride was added a catalytic amount of 4-dimethylaminopyridine and the resulting mixture was stirred overnight at room temperature. The reaction mixture was then poured into water, the layers partitioned and the aqueous layer was extracted twice with methylene chloride. The combined organic extracts were dried over $MgSO_4$ and concentrated to yield a yellow oil. Flash chromatography (elution with 15% ethyl acetate in hexane) gave 9.2 mg of the ester (140) as a rotameric mixture. ^1H NMR (500 MHz, $CDCl_3$) δ 8.01 (d), 7.94 (d), 7.59-7.52 (m), 7.46-7.39 (m), 7.19 (d), 7.12 (d), 6.82 (d), 6.51 (s), 6.38 (s), 5.43 (d), 4.78 (ABq), 4.62 (dd), 4.58 (s), 4.41 (d), 3.51 (dd), 3.23 (ddd), 3.01 (ddd), 2.41 (d), 2.24 (d), 1.91 (s), 1.84-1.76 (m), 1.65-1.48 (m), 0.96 (s), 0.18 (s).

7. (E)-3-[cis-4-(hydroxycyclohexyl)]-2-methylprop-2-enyl N-(phenylglyoxyl)-pipecolate (25)

To a solution of 9.2 mg (0.02 mmol) of ester (140) in 1.0 mL of acetonitrile at 25°C was added dropwise a solution consisting of a 95:5 mixture of acetonitrile: 48% hydrofluoric acid and the reaction was stirred until thin layer chromatography (TLC) indicated the disappearance of starting material. The reaction was

quenched by the addition of saturated potassium carbonate. The reaction mixture was extracted with three portions of ethyl acetate, dried over $MgSO_4$ and concentrated. Flash chromatography (elution with 35% ethyl acetate in hexane) yielded 6.1 mg (82%) of the ester (25) as a rotameric mixture. ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (dd), 7.97 (dd), 7.67-7.59 (m), 7.56-7.48 (m), 5.49 (d), 5.44 (d), 4.61 (ABq), 4.44 (s), 4.41 (d), 3.98-3.91 (m), 3.5 (br d), 3.26 (ddd), 3.11 (ddd), 2.43-2.18 (m), 1.86-1.37 (m), 1.72 (d), R_f 0.57 (3:1 ethyl acetate-hexane).

EXAMPLE 2

Synthesis of (S)-Benzyl N-(phenylglyoxyl)pipecolate (3)

To a solution of 43 mg (0.19 mmol) of freshly generated (S)-Benzyl pipecolate (133) (described in Example 1) in 2.0 mL of anhydrous methylene chloride was added 44 mg (0.29 mmol) of benzoylformic acid and 56 mg (0.29 mmol) of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and the resulting mixture was stirred overnight at room temperature. The reaction mixture was then poured into water, the layers partitioned and the aqueous layer was extracted twice with methylene chloride. The combined organic extracts were dried over $MgSO_4$ and concentrated to yield a yellow oil. Flash chromatography (elution with 1:3 ether-hexane) gave 49 mg (72%) of the keto-amide (3) as a rotameric mixture. ^1H NMR (500 MHz, $CDCl_3$) δ 7.98 (d), 7.91 (d), 7.58 (t), 7.41-7.30 (m), 5.45 (d), 5.21 (ABq), 5.06 (ABq), 4.61 (dd), 4.42 (d), 3.48 (dd), 3.19 (ddd), 2.96 (ddd), 2.40 (d), 2.21 (d), 1.83-1.72 (m), 1.61-1.49 (m), 1.46-1.33 (m), R_f 0.55 (1:1 ether-hexane).

EXAMPLE 3

Synthesis of (S)-Benzyl N-[(3-methoxyphenyl)glyoxyl)]-pipecolate (9)

The keto-amide (9) was prepared from 45 mg (0.205 mmol) of (S)-Benzyl pipecolate (133) (described in Example 1) and 55 mg (0.306 mmol) of 3-methoxybenzoyl-formic acid (Barnish, T. et al., J. Med. Chem. 24:339 (1981)) as described in Example 2. Flash chromatography (elution with 1:4 ether-hexane) gave 73 mg (93%) of (9) as a rotameric mixture. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59-7.10 (m), 5.42 (d), 5.23 (ABq), 5.09 (ABq), 4.59 (dd), 4.38 (d), 3.82 (s), 3.81 (s), 3.48-3.40 (m), 3.20 (ddd), 2.98 (ddd), 2.39 (d), 2.21 (d), 1.82-1.70 (m), 1.61-1.22 (m), R_f 0.45 (1:1 ether-hexane).

EXAMPLE 4

Synthesis of (S)-Benzyl N-(2-furylglyoxyl)pipecolate (20)

To a 0°C solution of 412 mg (1.03 mmol) of (S)-Benzyl pipecolate salt (134) (described in Example 1) in 40 mL of acetonitrile was added 198 μL (1.14 mmol) of diisopropylethylamine, 174 mg (1.24 mmol) of α-oxo-2-furanacetic acid, 579 mg (1.24 mmol) of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate and then 216 μL (1.24 mmol) of diisopropylethylamine. The resulting reaction mixture was stirred at ambient temperature for 14 h and then evaporated to dryness. The residue was dissolved into 150 mL of ethyl acetate, washed sequentially with 50 mL of 0.5 N HCl, 50 mL of saturated $NaHCO_3$, 50 mL of brine and was then dried over

$MgSO_4$ and concentrated. Flash chromatography (elution with 2% ether in methylene chloride) provided 163 mg (46%) of the keto-amide (20) as an oil. The ^1H NMR spectrum of this compound (300 MHz, $CDCl_3$) was consistent with the product as a mixture of rotamers. R_f 0.2 (2% ether in methylene chloride). HPLC, R_t=12.83 min.

EXAMPLE 5

Synthesis of (S)-Benzyl N-(4-Methoxycinnamoyl)pipecolate (112)

To a solution of 145 mg (0.37 mmol) of (S)-Benzyl pipecolate salt (134) (described in Example 1) in 8.0 mL of methylene chloride was added 102 mg (0.57 mmol) of 4-methoxycinnamic acid, 107 mg (0.55 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 130 μL (0.74 mmol) of diisopropylethylamine. The resulting solution was stirred at ambient temperature for 12 h and was then concentrated under reduced pressure. Flash chromatography (elution with 1:1 ethyl acetate-hexane) gave 91 mg (65%) of the amide (112) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.66 (d), 7.50 (d), 7.35 (m), 6.90 (d), 6.82 (d), 6.63 (d), 5.55 (d), 5.20 (br s), 4.86 (br s), 4.67 (br d) 4.03 (br d), 3.83 (s), 3.37 (dt), 2.78 (dt) 2.33 (br d), 1.74 (m), 1.43 (m). R_f 0.40 (1:1 ether-hexane).

EXAMPLE 6

Synthesis of (S)-3-(3,4-Methylenedioxyphenyl)-prop-2-enoylproline benzyl ester (109) and (S)-3-(3,4-Methylenedioxyphenyl)-prop-2-enoylalanine benzyl ester (132)

A solution of 192 mg (1.0 mmol) of 3,4-methylenedioxycinnamic acid, 121 mg (0.5 mmol) of proline benzyl ester hydrochloride and 108 mg (0.5 mmol) of alanine benzyl ester hydrochloride in 6 mL of acetonitrile was treated sequentially with 0.35 mL (2.0 mmol) of diisopropylethylamine and 443 mg (1.0 mmol) of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate. The mixture was stirred for 16 hrs then concentrated in vacuo. The residue was dissolved in 10 mL of dichloromethane and poured into 3 volumes of diethyl ether. The mixture was washed sequentially with water, 10% potassium bisulfate solution, water, saturated sodium bicarbonate solution, water and saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography, using a stepwise gradient of 30%, 35%, 40%, and 45% ethyl acetate in hexane as eluant. Ester (109) (142 mg) was obtained as a colorless oil, Rf = 0.4 (40% ethyl acetate/hexane); HPLC, R_t = 12.26; ^{1}H NMR (300 MHz) consistent with structure. Ester (132) (162 mg) was obtained as a colorless oil; TLC, R_f = 0.22 (40% ethyl acetate/hexane); HPLC, R_t = 11.84 min; ^{1}H NMR (300 MHz) consistent with structure.

EXAMPLE 7

Synthesis of (S)-N-3-(4-methoxyphenyl)-prop-2-enoyl-N-methylalanine benzyl ester (129)

1. (S)-N-Methylalanine benzyl ester p-toluene sulfonic acid salt (141)

A suspension of 1.55 g (15.0 mmol) of (S)-N-methylalanine and 9.31 (90.0 mmol) of benzyl alcohol in 30 mL of toluene was treated with 3.00 g (15.8 mmol) of p-toluenesulfonic acid monohydrate. The mixture was heated for 19 hours under reflux with removal of water via a Dean-Stark trap. After cooling, the reaction solution was poured into 200 mL of ether, precipitating a yellow oil. The solvents were decanted and the residue was taken up into ethyl acetate and concentrated to yield a viscous, light yellow oil (141); TLC: R_f = 0.34, 95:5:0.5 CH_2Cl_2/MeOH/concentrated ammonium hydroxide; ^1H NMR (300 MHz) consistent with structure.

2. (S)-N-3-(4-methoxyphenyl)-prop-2-enoyl-N-methylalanine benzyl ester (129)

A suspension of 96 mg (0.5 mmol) of 4-methoxycinnamic acid and 121 mg (0.5 mmol) of (141) in 6 mL of dichloromethane was cooled in an ice/water bath under nitrogen. The mixture was treated with 0.26 mL (1.5 mmol) of diisopropylethylamine and 135 mg (0.53 mmol) of N,N-bis-(2-oxo-3-oxazolidinyl) phosphinic chloride and then stirred for 16 hours, warming slowly to ambient temperature. The mixture was poured into three volumes of diethyl ether and washed sequentially with water, 10%

potassium bisulfate solution, water, saturated sodium bicarbonate solution, water, and saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by preparative thick layer silica gel chromatography using 40% ethyl acetate/hexane as eluant. Ester (129) (40 mg) was obtained as a yellow oil: TLC, R_f = 0.24 (35% ethyl acetate/hexane); HPLC, R_t = 13.34 min; ^1H NMR (300 MHz) consistent with structure.

EXAMPLE 8

Synthesis of (S)-N-Methyloxalyl-N-ethylalanine benzyl ester (127)

1. (S)-N-9-Fluorenylmethoxycarbonyl-N-ethylalanine benzylester (142)

A suspension of 848 mg (2.5 mmol) of (S)-N-Fmoc-N-ethylalanine in 10 mL of dichloromethane was treated with 436 μL (5.0 mmol) of oxalyl chloride followed by a catalytic amount (1 drop) of dimethylformamide. The mixture was stirred for one hour, then concentrated in vacuo. The yellow, oily residue was treated with 10 mL of toluene followed by 517 mg (0.5 mmol) of benzyl alcohol and 669 mg (5.0 mmol) of silver cyanide. The mixture was heated in an 80°C oil bath with vigorous stirring for 20 minutes, then cooled and filtered through a pad of diatomaceous earth. The filtrate was concentrated, and the residue was purified by silica gel column chromatography, using 10% acetone in hexane as eluant. Ester (142) (810 mg) was obtained as a colorless oil; TLC: R_f = 0.28, 15% acetone/hexane; ^1H NMR (300 MHz) consistent with structure.

2. (S)-N-Methyloxalyl-N-ethylalanine benzyl ester (127)

A solution of 0.25 g (0.58 mmol) of (142) in 3 mL of acetonitrile was treated with 3 mL of diethylamine and the mixture was allowed to stand for 20 min. The mixture was concentrated in vacuo and the residue was taken up in 10 mL of acetonitrile and again evaporated. After repeating this process, the residue was dissolved in 4 mL of dichloromethane, cooled in an ice/water bath under nitrogen, and treated with 111 μL (0.64 mmol) of diisopropylethylamine followed, during approximately 1 min. with 54 μL (0.64 mmol) of methyl oxalyl chloride. The mixture was stirred overnight, warming slowly to ambient temperature, then poured into three volumes of ether. The mixture was washed sequentially with water, 10% potassium bisulfate solution, water, saturated sodium bicarbonate solution, water, and saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography using 1:7 acetone: hexane as eluant. Ester (127) (147 mg) was obtained as a colorless oil, R_f = 0.36, 35% acetone/hexane; HPLC, R_t = 12.19 min; ^1H NMR (300 MHz) consistent with structure.

EXAMPLE 9

Synthesis of (S)-3-Cyclopentylpropyl N-(2-Methyloxalyl)-pipecolate (135)

1. (S)-N-(Methyloxalyl)pipecolic Acid (143)

The acid (143) was prepared from methyl oxalyl chloride as described in Example 1 for the production of

(S)-N-(Phenylglyoxyl)pipecolic acid (135). Thus, 3.16 g (11.32 mmol) of the tartrate salt of (S)-pipecolic acid and 1.19 mL (12.45 mmol) of methyl oxalyl chloride gave 1.25 g (51%) of the acid (143) as a tan solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31 (d), 4.62 (d), 4.49 (br d), 3.61 (br d), 3.90 (s), 3.88 (s), 3.46 (dt), 2.97 (dt), 2.40-1.40 (m).

2. (S)-3-Cyclopentylpropyl N-(2-Methyloxalyl)pipecolate (35)

The ester (35) was prepared from 3-cyclopentyl-propan-1-ol and the acid (143) as described in Example 2. Flash chromatography (elution with 2% ether in methylene chloride) gave 72 mg (48%) of (35) as a colorless oil. The ^{1}H NMR spectrum of this compound (300 MHz, $CDCl_3$) was consistent with the product as rotamers. R_f 0.56 (10% ether in methylene chloride). HPLC, R_t = 14.30 min.

DISCUSSION OF ASSAYS

Cell Source and Culture

Fresh peripheral blood lymphocytes (PBLs) from LeukoPak cells or whole blood from random normal blood donors (tested HIV-negative and hepatitis negative) are isolated and separated by density centrifugation over Histopaque 1077 (Sigma Chemical Co., St. Louis, MO). The murine CTLL cytotoxic T cell line and the human Jurkat T cell line are from ATCC (CTLL-2 ATCC TIB214, JURKAT CLONE E6-1 ATCC TIB152). The human allogeneic B cell lines used for activation of the fresh PBLs are EBV-transformed lymphocytes from normal healthy adult donors with two

completely different HLA haplotypes. All cell lines were routinely tested for the presence of Mycoplasma contamination using the Gibco Mycotect test kit and are Mycoplasma-free. Culture medium consists of RPMI 1640 (Gibco, Grand Island, NY) containing penicillin (50 U/ml) and streptomycin (50 μg/ml), L-glutamine 2 mM, 2 mercaptoethanol (5×10^{-5}), 10% heat-inactivated FCS and 10 mM HEPES.

Compound Solutions and Titrations

All chemical stocks were dissolved in DMSO. Titrations of compounds were made into the medium the individual assay was carried out in, i.e., complete RPMI or HB 104 for final diluted concentrations, using multiple three-fold dilutions from 1 μM or 10 μM stock solutions.

MTT Assay

The MTT assay is a colorimetric technique to determine the toxicity of the compounds on growing lymphoid and non-lymphoid cell lines based on reduction of the tetrazolium salt by intact mitochondria (Mossman, T., J. Immunol. Methods 65:55 (1983)). Cell viability in the presence or absence of different concentrations of test compounds in serum-free medium (HB 104, HANA Biologic, Inc.) was assessed using MTT (3-[4,5-dimethyl-thiazoyl-2-yl]2,5-diphenyl-tetrazolium bromide). At 4 h before the end of the 3-day toxicity assay culture period, 20 μl of MTT dye (5 mg/ml in pH 7.2 PBS) were added to each microtiter well. At the end of the incubation time, most of the culture media was carefully aspirated out of each

well. Then 100 μl of acidified isopropyl alcohol (0.04 N HCl) was added to solubilize the dye and optical density is read at 570 nm minus OD at 630 nm (Molecular Devices Thermomax plate reader and Softmax software program, Menlo Park, CA). Results were compared with mean OD in controls (medium with no drugs) and doses causing 50% toxicity (TC_{50}) were calculated.

Mitogenesis Assays ("PMA" and "OKT3")

The inhibitory effect of test compounds on the proliferation of human PBLs in response to mitogens (Waithe, W.K. and K. Hirschhorn, Handbook of Experimental Immunology, 3d Ed. Blackwell Scientific Publications, Oxford (1978); Mishell, B.B. and S.M. Shiigi, Selected Methods in Cellular Immunology W.H. Freeman and Co., San Francisco, CA (1980)) was assessed by stimulation of 5 x 10^4 cells with OKT3 (10^{-4} dilution final) or PMA (10ng/ml) plus ionomycin (250 ng/ml) in the presence or absence of different concentrations of test compounds and control drugs (CsA, FK506, Pagamycin) in final volume of 200 μl per well in 96 well round bottomed plates. After 48 h incubation (37°C, 5% CO_2), cells were pulsed with 1 μCi of ^{3}H-thymidine, harvested 24 h later with a Tom Tek cell harvester, and counted in LKB β-scintillation counter. Results (cpm) were compared with controls with medium alone, and concentrations causing 50% reduction in counts (IC_{50}) were calculated.

MLR Bioassays ("LB" and "JVM")

Antigen activated proliferation of PBLs in a primary mixed lymphocyte reaction was assessed in the presence or

absence of different concentrations of tested compounds and control drugs. 5×10^4 fresh PBLs were stimulated with 5×10^3 of Mitomycin C treated-allogeneic EBV-transformed β-lymphoglastoid cells, LB and JVM, in a final volume of 200 μl per well in 96-well round-bottomed plates (Mishell, B.B. and S.M. Shiigi, Selected Methods in Cellular Immunology W.H. Freeman and Co., San Francisco, CA (1980); Nelson, P.A. et al., Transplantation 50:286 (1990)). Cultures were pulsed on day 6, harvested 24 h later and counted as in previous section.

IL-2 Microassay ("CTLL")

To determine if test compounds inhibit the later T cell activation process of cytokine utilization, the proliferative response of the IL-2 dependent CTLL-20 murine T cell line (ATCC) was assessed (Gillis, S. et al., J. Immunology 120:2027 (1978)). CsA and FK506 inhibit the production of IL-2 by activated T cells, whereas Rapamycin interferes with the utilization of IL-2. Rapamycin thus inhibits IL-2 dependent proliferation of the CTLLs, and CsA and FK506 do not (Dumont, F.J. et al., J. Immunology 144:251 (1990)). 3×10^3 CTLLs were exposed to different concentrations of test compounds and control drugs in the presence of 1 U/ml of human recombinant IL-2 (Genzyme, rIL-2) for 24 h. Four h after adding drugs, cells were pulsed with 1 μCI of 3H-thymidine, incubated for an additional 20 h (37°C, 5% CO_2), and then harvested and counted as previously described.

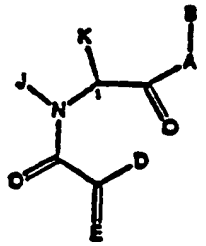
CLAIMS

1. An immunosuppressant compound having an affinity for FK-506 binding protein and a molecular weight below about 750 amu.

2. An immunosuppressant compound of Claim 1, capable of inhibiting the prolyl peptidyl cis-trans isomerase activity of the FK-506 binding protein.

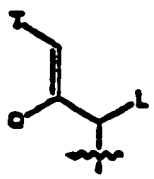
3. An immunosuppressant compound of Claim 1, having a molecular weight below about 500 amu.

4. A compound having immunosuppressive activity, represented by the formula:



and pharmaceutically acceptable salts thereof, wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B is hydrogen, CHL-Ar, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl or Ar substituted (C1-C6)-alkyl or alkenyl, or



wherein L and Q are independently hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, CF_3, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino and phenyl;

wherein D is either hydrogen or U; E is either oxygen or CH-U, provided that if D is hydrogen, then E is CH-U or if E is oxygen then D is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl or (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl (C1-C4)-straight or branched or alkenyl, 2-indolyl, 3-indolyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar;

wherein J is hydrogen or C1 or C2 alkyl; K is (C1-C4)-straight or branched alkyl, benzyl or

cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an O, S, SO or SO_2 substituent therein, and

wherein the stereochemistry at carbon position 1 is R or S.

5. An immunosuppressant compound of Claim 4, having an affinity for FK-506 binding protein.

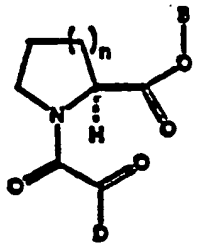
6. An immunosuppressant compound of Claim 4, capable of inhibiting the prolyl peptidyl cis-trans isomerase activity of the FK-506 binding protein.

7. An immunosuppressant compound of Claim 4, having a molecular weight below about 750 amu.

8. An immunosuppressant compound of Claim 7, having a molecular weight below about 500 amu.

9. An immunosuppressant compound of Claim 4, wherein the stereochemistry at carbon position 1 is S.

10. An immunosuppressant compound of Claim 4, wherein J and K are taken together and is represented by the formula:

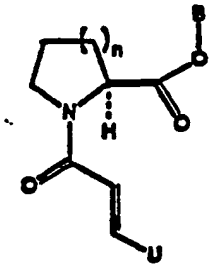


wherein n is 1 or 2.

11. An immunosuppressant compound of Claim 10, wherein B is selected from the group consisting of benzyl, alkyl, naphthyl, tert-butyl, hydrogen, E-3-phenyl-2-methyl-prop-2-enyl, E-3-(4-hydroxyphenyl)-2-methyl-prop-2-enyl, E-3-(4-hydroxycyclohexyl)-2-methyl-prop-2-enyl, cyclohexylethyl, cyclohexyl-propyl, S-sec-phenethyl, cyclohexylbutyl, cyclo-pentylpropyl, E-3-(4-methoxyphenyl)-2-methyl-prop-2-enyl, E-3-(3,4-dimethoxyphenyl)-2-methyl-prop-2-enyl and E-3-[cis-(4-hydroxycyclohexyl)]-2-methyl-prop-2- enyl; and

D is selected from the group consisting of phenyl, methoxyphenyl, cyclohexyl, ethyl, methoxy, nitrobenzyl, thiophenyl, indolyl, furyl, pyridyl, pyridyl-N-oxide, nitrophenyl, fluorophenyl, trimethoxyphenyl and dimethoxyphenyl.

12. An immunosuppressant compound of Claim 4, wherein J and K are taken together and is represented by the formula:



wherein n is 1 or 2.

13. An immunosuppressant compound of Claim 12, wherein U is selected from the group consisting of methoxyphenyl, hydrogen, dimethoxyphenyl, trimethoxyphenyl, dimethylaminophenyl, nitrophenyl, furyl, indolyl, pyridyl and methylenedioxyphenyl.

14. A compound having immunosuppressive activity represented by any of the structures shown in Figure 1 and having an affinity for FK-506 binding protein.

15. A composition for suppressing an immune response in an individual, comprising an immunosuppressive amount of an immunosuppressant compound having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu, in a physiologically acceptable vehicle.

16. A composition of Claim 15, wherein the immune reponse to be suppressed is an autoimmune response.

17. A composition of Claim 15, wherein the immune response to be suppressed is an immune response associated with graft rejection.

18. A composition of Claim 15, further comprising an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK-506, 15-deoxyspergualin, OKT3 and azathioprine.

19. A composition of Claim 15, further comprising a steroid.

20. A composition for suppressing an immune response in an individual, comprising an immunosuppressive amount of a compound of Claim 4 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu, in a physiologically acceptable vehicle.

21. A composition of Claim 20, wherein the immunosuppressant compound is represented by any of the compounds recited in Tables 1, 2, 3 or 4.

22. A composition of Claim 20, wherein the immunosuppressant compound is represented by the structures shown in Figure 1.

23. A composition of Claim 20, wherein the immune reponse to be suppressed is an autoimmune response.

24. A composition of Claim 20, wherein the immune response to be suppressed is an immune response associated with graft rejection.

25. A composition for preventing or significantly reducing graft rejection in a bone marrow or organ transplant in an individual, comprising an immunosuppressive amount of a compound of Claim 4 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu, in a physiologically acceptable vehicle.

26. A composition of Claim 25, wherein the immunosuppressant compound is represented by the structures shown in Figure 1.

27. A composition of Claim 25, further comprising an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK506, 15-deoxyspergualin, OKT3 and azathioprine.

28. A composition of Claim 15, further comprising a steroid.

29. A composition of preventing or significantly reducing an autoimmune response in a mammal, comprising an immunosuppressive amount of a compound of Claim 4 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu, in a physiologically acceptable vehicle.

30. A composition of Claim 29, wherein the immunosuppressant compound is represented by the structures shown in Figure 1.

31. A composition of Claim 29, wherein the mammal is a human.

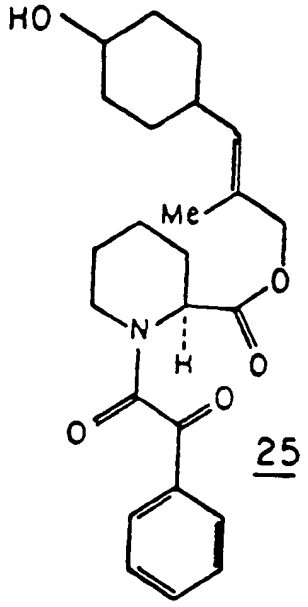


FIG. IA

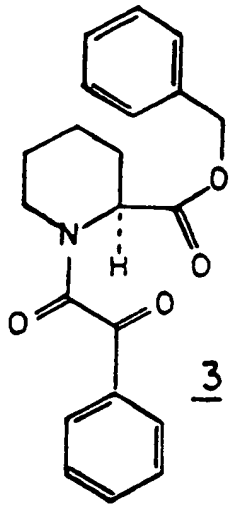


FIG. IB

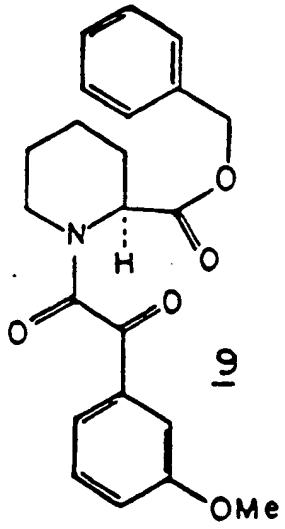


FIG. IC

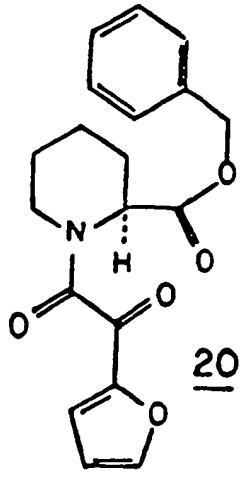


FIG. ID

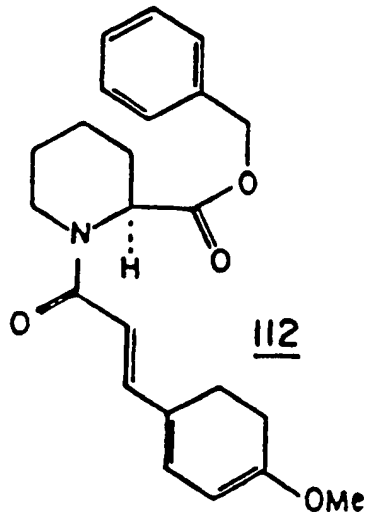


FIG. IE

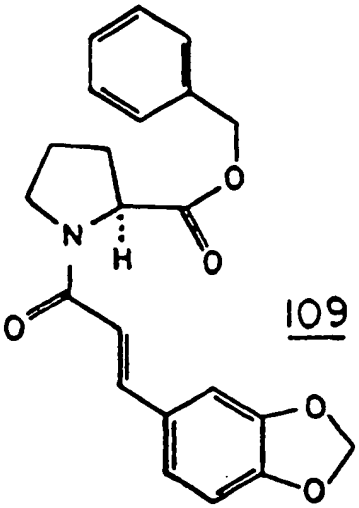


FIG. IF

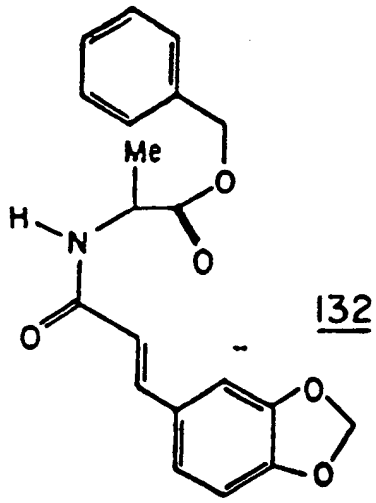


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FIG. 1G

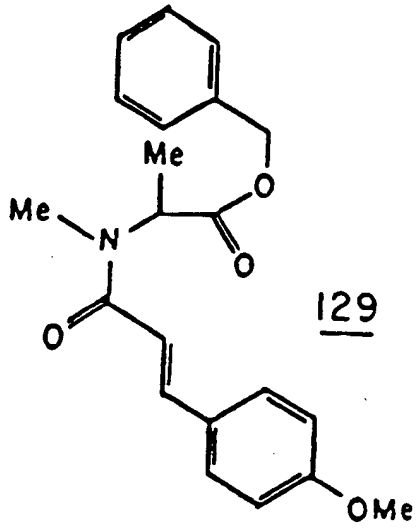

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FIG. 1H

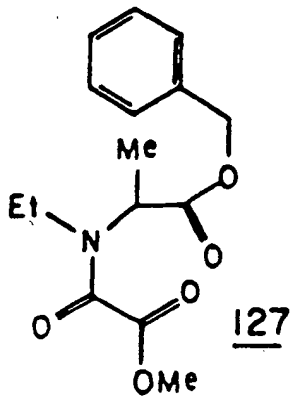

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FIG. 1I

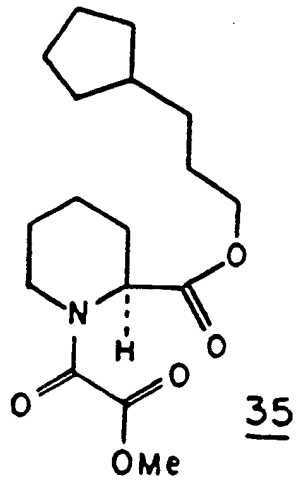

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FIG. 1J

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/04694

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 D 211/60, 207/16, 401/06, 405/06, 409/06, 317/60
C 07 C 235/72, A 61 K 31/395, 31/215, 31/335

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Chemical Abstracts, vol. 112, no. 6, 5 February 1990, (Columbus, Ohio, US), Boulmedais A. et al: "Stereochemistry of electrochemical reduction of optically active alfa-ketoamides. II. Electro-reduction of benzoylformamides derived from S-(-)--proline", page 568, abstract 44173u, & Bull. Soc. Chim. Fr. 1989, (2), 185-91, see reg. no. 94726-50-8 and 124778-23-0	11
X	Chemical Abstracts, vol. 103, no. 9, 2 September 1985, (Columbus, Ohio, US), Soai K. et al: "Diastereoselective reduction of chiral alpha--ketoamides derived from (S)-proline esters with sodium borohydride. Preparation of optically active alpha-hydroxy acids", page 614, abstract 70915w, & J. Chem. Soc., Perkin Trans. 1 1985, (4), 769-72, see reg.no. 97409-49-9 and 97409-50-2	11

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th October 1991	08. 11. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Danielle van der Haas

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, vol. 106, no. 25, 22 June 1987, (Columbus, Ohio, US), Nestor J. J., "N,N-dialkyl-guanidino dipeptides as antihypertensives", page 710, abstract 214389q, & EurPat. Appl. EP 196,841, see reg.no. 108415-25-4	11
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X	Chemical Abstracts, vol. 110, no. 7, 13 February 1989, (Columbus, Ohio, US), Egbertson M. el al: "A synthetic route to the tricarbonyl region of FK-506", page 659, abstract 57371r, & J. Org. Chem. 1989, 54(1), 11-12, see reg.no.118143-77-4	11
	--	
X	Chemical Abstracts, vol. 112, no. 1, 1 January 1990, (Columbus, Ohio, US), R.S. Coleman et al: "Degradation and manipulations of the immunosuppressant FK506: preparation of potential synthetic intermediates", page 707, abstract 7219t, & Heterocycles 1989, 28(1), 157-61, see reg.no. 124093-26-1	11
	--	
X,P	Science, vol. 249, 20 July 1990, R. W. Finberg et al: "Prevention of HIV-1 Infection and Preservation of CD4 Function by the Binding of CPFs to gp120", pages 287-291, see fig. 1	11
	--	
X	DE, A, 2328391 (DEUTSCHE GOLD- UND SILBER-SCHEIDEANSTALT VORMALS ROESSLER) 10 January 1974, see example 8	13
	--	
X	Chemical Abstracts, vol. 85, no. 18, 1 November 1976, (Columbus, Ohio, US), Kaczmar B.U. et al: "Snake-cage polymers, 1. Synthesis of various snake-cage polyelectrolytes consisting of polyacrylamides and an anion exchanger", page 26, abstract 124738w, & Makromol. Chem. 1976, 177(7), 1981-9, see reg.no. 60460-30-2	13
	--	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, vol. 85, no. 12, 20 September 1976, (Columbus, Ohio, US), Blaschke G. et al: "Investigation of chromatographic resolutions of racemates, VI. Polymeric amino acids derivatives as optically active adsorbents", page 3, abstract 78405k, & Chem. Ber. 1976, 109(6), 1967-75, see reg.no. 59977-03-6	13
	--	
X	Chemical Abstracts, vol. 110, no. 17, 24 April 1989, (Columbus, Ohio, US), Effenberger F. et al: "Amino Acids. 14. Diastereoselective addition of benzenesulfenyl chloride to 1-acryloylproline esters", page 778, abstract 154847h, & Chem. Ber. 1989, 122(3), 545-51, see reg.no. 86711-03-7, 117775-65-2, 118335-66-3, 118335-67-4 and 118335-84-5	13
	--	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [ ] Claim numbers ..... because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claim numbers 1-10, 12, 15-31 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

These claims do not clearly define the matter for which protection is sought, see Article 6.

3. [ ] Claim numbers ..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 91/04694

SA 49778

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 27/09/91
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A- 2328391	10/01/74	BE-A-	801089	18/12/73
		FR-A-	2189030	25/01/74
		JP-A-	49061136	13/06/74
		NL-A-	7308432	21/12/73

For more details about this annex : see Official Journal of the European patent Office, No. 12/82 .

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